

WHAT IS CLAIMED IS:

1. A method for purifying adenovirus from contaminants in a sample pool, comprising:

5 contacting the sample pool with a hydroxyapatite chromatographic medium to reversibly bind the adenovirus to the hydroxyapatite; and
 eluting the bound adenovirus from the hydroxyapatite.

10 2. The method of claim 1, wherein the sample pool comprises sodium chloride in a concentration of from about 150 to 500 mM.

15 3. The method of claim 1, wherein the hydroxyapatite chromatographic medium is equilibrated with a buffer comprising sodium chloride at a concentration of from about 150 to 500 mM before the step of contacting the sample pool with the hydroxyapatite.

20 4. The method of claim 1, further comprising the step of washing the hydroxyapatite with a buffer comprising sodium chloride in a concentration of 150 to 500 mM, wherein the hydroxyapatite comprises an adenovirus bound thereto.

25 5. The method of claim 1, wherein the adenovirus is eluted using a buffer comprising sodium chloride in a concentration from about 150 mM to 500 mM.

 6. The method of claim 1 wherein the sodium chloride concentration in a buffer used in the method is from about 350 mM to 450 mM.

 7. The method of claim 1, wherein the sample pool is prepared from an eluate of a conventional chromatography medium.

8. The method of claim 7 wherein the conventional chromatography medium is:
an anion exchange resin;
an immobilized metal ion affinity resin;
a size exclusion chromatography resin; or
a medium used in hydrophobic interaction chromatography.

9. The method of claim 1, wherein the eluting step is a gradient elution to 600 mM phosphate.

10. The method of claim 1, wherein the eluting step is a step elution with 250 mM phosphate.

11. The method of claim 1, wherein a buffer used in the method comprises glycerol or sucrose.

12. The method of claim 1, wherein the concentration of adenovirus in the sample pool is equal to or less than 1×10^{14} particles per ml.

13. The method of claim 1, wherein the adenovirus comprises a therapeutic gene.

14. The method of claim 1, wherein the adenovirus is ACN53.

15. The method of claim 1, wherein the adenovirus comprises a nucleic acid sequence from the p53 gene or from the p21 gene.

16. A method of claim 1, which reduces the concentration of a contaminant in the sample pool by at least 80%.

17. A method of claim 1, which reduces the concentration of empty capsids by at least 75%.

18. A method of claim 1, which reduces the concentration of BSA by at least 70%.

19. A method for purifying adenovirus from contaminants in sample pool, comprising:

contacting the sample pool with a hydroxyapatite chromatographic medium to reversibly bind the adenovirus to the hydroxyapatite;

5 washing the adenovirus-bound hydroxyapatite with a buffered solution; and

eluting the bound adenovirus from the hydroxyapatite,

wherein the sample pool is a buffered solution comprising about 50 mM sodium phosphate pH about 7.5, about 400 mM sodium chloride, about 2% sucrose, about 2 mM $MgCl_2$, and about 10% glycerol, and the concentration of total contaminants is reduced by at least 80%.

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100